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(72) Inventor; and
(71) Applicant : **GHISLAIN, Lucien** [US/US]; 1380 Millbrae Ave, Millbrae, California 94030 (US).

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(54) **Title**: APERTURE FORCE SENSOR

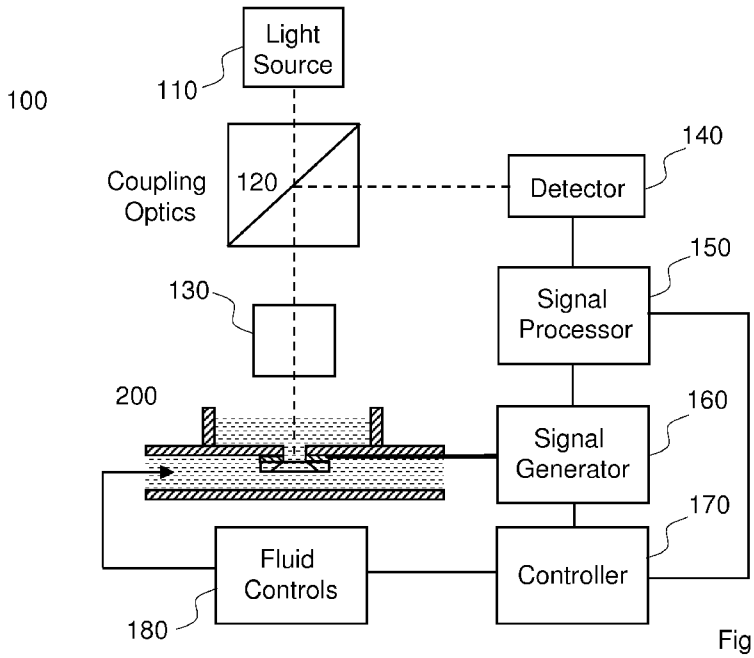

Fig. 1A

(57) **Abstract**: A sensor apparatus and method includes a sensor cell with a membrane having at least one aperture. The membrane includes a sensing surface and deflections of the membrane sensing surface are monitored with a membrane deflection sensor. In operation, analyte in a sample interacts with an aperture in the membrane to generate a force that changes membrane deflection. Analyte interaction with the aperture may be due to any of a wide variety of forces. The aperture may also be functionalized to detect a specific analyte interaction. The invention includes a method for detecting, monitoring, analyzing and modifying analyte in a sample, including detecting the presence of analyte, the amount of analyte or the rate of association and/or dissociation of the analyte with a binding partner.

APERTURE FORCE SENSOR

FIELD OF THE INVENTION

The invention relates to an apparatus and method for detecting, analyzing, monitoring, modifying analyte in a sample and in particular to an aperture force sensing device including a membrane with at least one aperture where the interaction of analyte with an aperture generates a force that is detected by a force sensor.

BACKGROUND OF THE INVENTION

There is extensive prior art on the measurement of small interaction forces between a probe and a sample surface. Scanning probe microscopes comprise a class of devices and methods with sensitivity down to the atomic level that includes at least two prominent members: the Scanning Tunneling Microscope (STM), U.S. Patent 4,343,993, developed by G. Binnig and H. Rohrer in 1981, and the Atomic Force Microscope (AFM), US Patent 4,724,318 by G. Binnig, C. Quate and Ch. Gerber in 1986. The Scanning Tunneling Microscope (STM) is designed to detect and image currents tunneling between an

electrically-conductive probe-tip and a sample surface and with atomic resolution. The Atomic Force Microscope (AFM) typically utilizes a microfabricated cantilever with integral probe-tip to measure tip-sample interaction forces and to image and study a wide range of surface properties with spatial resolution that can approach the atomic level. Forces measured by the AFM include but are not limited to: mechanical forces, steric forces, van der Waals forces, dipole-dipole forces, capillary forces, chemical forces, molecular forces, atomic forces, electric forces, capacitive forces, magnetic forces, hydrodynamic forces, viscous forces, frictional forces, dissipative forces, Casimir forces, solvation forces, thermal forces, optical forces.

The Atomic Force Microscope is a commercially successful instrument used for research, development and manufacturing due at least in part to its unique capabilities of imaging both electrically conductive and insulating samples and detecting very small forces. As evidence of the versatility of the atomic force microscope there are multiple cantilever-based imaging-modes such as contact-mode and tapping-mode as well as sensing-modes where additional quantities may be simultaneously or sequentially measured through the use of specialized probes, including but not limited to: magnetic force microscopy (MFM), electric force microcopy (EFM), chemical force microscopy (CFM), scanning capacitance microscopy (SCM), conductive atomic force microscopy (CAFM), tunneling atomic force microscopy (TUNA), scanning thermal microscopy (SThM), scanning voltage microscopy, scanning joule expansion microscopy, near-field scanning optical microscopy (NSOM), photothermal microspectroscopy and more.

Thus it is apparent that cantilever-based sensing of tip-sample interaction force measured based on the deflection of a cantilever is well established in the prior art.

There is also extensive prior art on the use of an aperture to detect and analyze analyte in a sample. The Coulter Counter, U.S. Patent 2,656,508 issued in 1953 is a device for detecting and counting particles in a fluid medium. Inventor Wallace H. Coulter is credited with the “Coulter Principle” which states that particles moving through an orifice

simultaneous with an electric current produce a change in impedance that is proportional to the volume of the particle traversing the orifice. When a particle enters the sensing zone it displaces a volume of electrolyte equivalent to the immersed volume of the particle and this causes a short-term change in the impedance across the aperture. The change in impedances can be measured as a resistive pulse; the height of each pulse being proportional to the volume of the particle. If constant particle density is assumed, the pulse height is also proportional to the particle mass. Pulse duration provides a measure of translocation time. Pulse frequency provides a measure of particle concentration.

The sensitivity of a resistive-pulse sensor, that is the smallest particle volume that can be detected, is largely limited by the diameter of the aperture or pore, though the length of the pore, the applied voltage, and the noise of the current trace also has an effect. Initially, resistive-pulse sensing was mainly used for examining bacteria and cells since the available commercial instruments could only detect particles larger than ~500 nm. In 1994, the applicable length scale of resistive pulse sensing was drastically decreased when Bezrukov et al. successfully demonstrated a biological nanopore effectively utilizing an ion channel as a molecular Coulter counter. In their device, two reservoirs were separated by a lipid bilayer into which an ion channel inserted itself. Measurements of the current through the nanometer-scale opening in the ion channel revealed the passage of single molecules contained in the solution. The use of a biological ion channel introduced analyte specificity to resistive pulse sensing because ion channels have functional chemical groups on their interiors that provide opportunities for chemical interaction between the particles and the pore.

The STM is primarily a current-sensing device, and generally does not offer the versatility of the AFM for sensing a wide range of interaction forces and operating in multiple modes. The aperture sensing prior art is primarily based on analyte detection using an electrical signal in the form of a resistive pulse that is associated with a current flowing through an

aperture. Similar to the case of the STM and AFM, prior art resistive pulse sensing generally does not offer the versatility that comes with sensing of interaction forces.

SUMMARY OF THE INVENTION

Glossary of Terms:

Analyte: a substance or material to be analyzed.

Aperture: a usually circular (but in general an arbitrary shape) and possibly variable and adjustable opening, hole, gap, constriction or pore that extends through a membrane. Aperture may form a conical facing up or down, a cylindrical, an hourglass shape going through the membrane or may have more complex shape and structure. In a preferred embodiment aperture size in a solid state membrane is fixed and determined by the fabrication process. In alternate embodiments the membrane is formed from flexible materials and aperture size is variable. Apertures may also include biological components such a lipid bilayers along with ion channels, for example, alpha-haemolysin.

Aperture size: The aperture size is measured by the effective aperture diameter and may be used to determine and control the flow of sample and analyte from one side of the membrane to the other. In the case where aperture diameter is greater than analyte size the invention allows for sensing aperture interaction forces due to multiple analytes. In the case where aperture diameter is greater than but comparable to analyte size the invention allows for sensing aperture interaction forces due to one or just a few analytes. In the case where aperture diameter is sufficiently small the invention allows for sensing aperture interaction forces due to just one analyte at a time. Biological apertures, such as ion channels, and microfabricated solid-state apertures can have diameters that are extremely small, extending down to the level of single molecules. For optimum sensitivity, the effective analyte size is typically comparable to the aperture size, analyte size may range from 1 mm (10^-3 meters) down to single molecules (10^-10 meters).

Deflection: a change in the position or movement. In the context of the present invention a force applied to the membrane generates a deflection that can typically be described by a

relation F ~ k Delta z, where F is the applied force, Delta Z is the membrane deflection and k is the effective spring constant of the membrane.

Electrokinetic flow: particle or fluid transport produced by an electric field acting on a fluid having a net mobile charge. Particles can be solid, liquid or gas bubbles with sizes on the scale of a micrometer and extending down to the molecular level. The common source of all these effects is the so-called interfacial 'double layer' of charges.

Electrophoresis: is the motion of particles under influence of electric field. It is ultimately caused by the presence of a charged interface between the particle surface and the surrounding fluid. In a conduction sample fluid an ion current may be generated by applying a bias voltage to electrodes immersed in the sample fluid in each of the two reservoirs. This sets up an electric field at the membrane aperture and also an electric current due to the flow of ions in solution, also known as an electrophoretic flow.

Electro-osmosis: is the motion of liquid in a porous body under influence of an electric field. Electro-osmotic flow is caused by the Coulomb force induced by an electric field on net mobile electric charge in a solution. Because the chemical equilibrium between a solid surface and an electrolyte solution typically leads to the interface acquiring a net fixed electrical charge, a layer of mobile ions, known as an electrical double layer or Debye layer, forms in the region near the interface. When an electric field is applied to the fluid (usually via electrodes placed at inlets and outlets), the net charge in the electrical double layer is induced to move by the resulting Coulomb force.

Flow: Motion of a fluid subjected to unbalanced forces or stresses. The motion continues as long as unbalanced forces are applied. A fluid may be a liquid, vapor, or gas. The term vapor denotes a gaseous substance interacting with its own liquid phase, for example, steam above water. If this phase interaction is not important, the vapor is simply termed a gas.

Force: is commonly known as a "push" or "pull," but more properly defined in physics as a quantity that changes the motion, size, or shape of a body. Force is a vector quantity, having both magnitude and direction. The magnitude of a force is measured in units such

as the Pound, Dyne, and Newton, depending upon the system of measurement being used. An unbalanced force acting on a body free to move will change the motion of the body.

Interaction force: is a kind of action that occurs as two or more objects have an effect upon one another tending to change the motion of the objects or produce motion or stress in a stationary object. Interaction forces relevant to the present invention include but are not limited to mechanical, inertial, viscous, hydrodynamic, frictional, lubrication, adhesive, dissipative, van der Waals, Casimir, ion correlation, salvation, hydrophobic, atomic, chemical, molecular, electrostatic, electric, magnetic, forces due to thermal effects such as thermal expansion and thermal gradients, optical forces, osmotic repulsion, double layer, thermal fluctuation, steric force, undulation force and protrusion force, capillary force. Interaction force is a quantitative description of the interaction between two physical bodies, such as an aperture in a membrane and sample fluid flowing through the aperture. Interaction forces may be near or below the microNewton level (10^-6 Newtons), in some cases interaction forces will be in the picoNewton (10^-12 Newton) range, and interaction forces may possibly extend down to the femto-Newton level (~ 10^-15 Newtons). Small interaction forces on the membrane generate correspondingly small deflections of the membrane which may be detected by use of a sensitive membrane deflection sensor.

Force modulation: change or variation of the force as a function of time. The modulation may be in the form of a ramp increasing or decreasing with time, it may be in the form of a pulse with a defined amplitude, duration and duty cycle, it may be in the form of a periodic oscillation with time or may in general have any more complex functional time dependence.

Membrane: A thin pliable sheet or skin formed of various materials supported at the perimeter but otherwise free to move and free to deflect under applied force. Also a thin sheet of material forming a partition that divides, separates and defines the boundary between two reservoirs. The membrane shape may be circular, elliptical, square, rectangular, polygonal, may in general have any suitable shape. The membrane may be formed from one or more layers and may include a sensing layer to provide for membrane deflection sensing. The membrane may be fabricated from solid state materials including

but not limited to silicon, silicon nitride, and silicon dioxide. The membrane may also be formed from biological molecules including but not limited to lipid bilayers. In addition the membrane may be formed from polymeric and elastomeric materials. In a preferred embodiment the membrane includes one aperture, in alternate embodiment the membrane includes multiple apertures. The membrane thickness is preferably in the range 1 to 100 nanometers (1 nanometer = 10^-9 meters). In alternate embodiments the membrane thickness may extend down to the atomic level ~ 0.1 nanometer (10^-10 meter) and in still further embodiments the membrane thickness may extend up to the millimeter level (1 millimeter = 10^-3 meters). In a preferred embodiment the membrane size is in the range 1 to 100 microns (1 micron = 10^-6 meter) and in alternate embodiments the membrane size may extend down to 10 nanometers (10^-8 meters) and up to 10 centimeters (10^-1 meters). Smaller, thinner membranes generally provide higher sensitivity.

Membrane deflection sensor: a device that detects membrane deflection and turns it into a signal which can be measured or recorded. Deflection sensors can operate based on a variety of physical principles and effects including but not limited to: optical interference, optical lever beam deflection, piezo-electric effects, piezo-resistive effects, capacitive effects, electromagnetic effects, electronic effects. In a preferred embodiment the aperture force sensing device includes a membrane deflection sensor that is based on optical interferometry which is known from prior art to be capable of detecting very small displacements.

Pressure driven flow: Fluid flow through an aperture may be generated by applying pressure to one of the reservoirs so that there is a pressure gradient at the at least one aperture. The volumetric flow rate Q of a fluid with viscosity n due to a pressure difference Delta P applied across a cylindrical aperture of length L and radius R is given by the Hagen-Poiseuille equation:

Q = pi (R)^4 Delta P / 8 L n

Thus for a cylindrical aperture the volumetric flow rate varies as the fourth power of the aperture radius. It can also be shown using the Hagen-Poiseuille equation that the

volumetric flow rate for a conical aperture varies as the third power of the aperture radius (R^3).

Reservoir: a chamber of a sample cell. The two chambers of the sample cell are designated the Cis reservoir and the Trans reservoir.

Sample: A representative fraction of material tested or analyzed in order to determine the nature, composition, activity, function and/or percentage of specified constituents. In a preferred embodiment the sample is a conductive fluid that carries analyte.

Sample cell: a support for the membrane including at least two fluid reservoirs separated by a boundary with the membrane defining at least part of the boundary such that flow of sample and analyte between the fluid reservoirs can occur only by translocation through an aperture in the membrane. The sample and analyte are introduced in the Cis reservoir and translocate through a membrane aperture to the Trans reservoir.

Specific binding: A specific binding reaction is by definition a saturable reaction, usually reversible, that can be competed by an excess of one of the reactants. Specific binding reactions are characterized by a complementarity of shape, charge and other binding determinants as between the participants in the specific binding reaction. The analyte and anti-analyte molecules may be members of a binding pair, examples include: antibodies, DNA, RNA, protein, small and large molecules, lipids, monolayer and bilayer membranes, cells, receptors and their binding targets, aptamers, biomarkers, toxins.

Translocation: the act, process, or an instance of changing location or position. The flow of sample and analyte through an aperture from the Cis to the Trans reservoirs of the sample cell is defined to be translocation of the sample and analyte.

The present invention is a versatile device and method that may be optimized to detect, monitor and analyze and modify analyte in a sample and where any one or more of a wide range of interaction forces between analyte and an aperture in a membrane can be studied. In some cases the device and method is capable of operating with molecular resolution. The key principle is that flow or motion of analyte and sample through a membrane aperture allows for analyte interaction with the aperture to generate interaction forces that

result in a change of the force on the membrane and a change in membrane deflection. In analogy to Atomic Force Microscopy the method of the invention may be called Aperture Force Sensing and the device may be called an Aperture Force Sensor. The methods of the invention may be used to detect the presence of one or more analytes in a sample, to analyze characteristics of one or more analytes in a sample, to continuously monitor analyte interaction over a period of time and even to modify analyte.

According to a preferred embodiment of the invention the device includes, but is not limited to: A membrane including at least one aperture exposed to a sample and analyte. A sample cell including cis and trans reservoirs with the membrane mounted and sealed such that it defines the boundary between the two reservoirs. A sample cell mount with precision adjusters to align the membrane and optimize the membrane deflection sensor signal. A sample cell transducer: in one embodiment the transducer generates a membrane driving force; in an alternate embodiment the transducer generates a driving force on sample and analyte at or near the aperture. A membrane deflection sensor based on optical interferometry that includes a membrane sensing surface, a sensor element including a reference surface and a detector. Fluidic connections and controls including pumps to provide for sample handling to and from the sample cell. Electrodes immersed in the sample cell reservoirs and connected to a potentiostat to drive electrokinetic flows. A signal generator to provide a driving signal to the sample cell transducer. A signal processor including a phase sensitive amplifier and capable of measuring both the amplitude and phase of periodic signals. The deflection sensor signal provides an input and the signal generator provides a reference to the signal processor with the signal processor output directed to a system controller. The system controller controls each of the subsystems with software interface.

According to a preferred embodiment of the invention the method is characterized by, but not limited to the following steps: a membrane including at least one aperture is mounted and aligned in a sample cell and exposed to sample and analyte. The flow of sample and

analyte through the aperture may be driven by electrokinetic effects such as electrophoresis and electro-osmosis using the electrodes immersed in the sample cell reservoirs and with a bias voltage applied by a potentiostat; the flow may also be pressure driven and directed by fluid controls. Analyte interaction with the aperture may also occur without fluid flow by allowing the analyte to passively diffuse to the membrane aperture. The presence of analyte in the aperture generates an interaction force that changes the deflection of the membrane. The membrane deflection sensor detects membrane deflection as an optical interference signal between light reflected by the membrane sensing surface and the sensor element; the detector converts the optical signal to an electrical signal that is directed to the signal processor, and the system controller monitors and records the events to enable the detection, monitoring, counting and analysis of analyte interaction with the membrane aperture. To operate in a modulation mode, the system controller directs the signal generator to produce a driving signal that is directed to the sample cell transducer which applies a driving force to the membrane; alternately the transducer applies a driving force to sample and analyte. The signal processor accepts the driving signal and the membrane deflection signal as inputs and provides an output signal to the system controller that measures both amplitude and phase of the membrane motion. The system controller also directs fluid controls to adjust the flow of sample and analyte in order to optimize the detection and analysis. The fluid controls may also control the concentration of analyte in the sample to optimize the detection and analysis.

In another embodiment of the method of the invention the aperture force sensor operates simultaneously or sequentially with a resistive pulse sensor. In this mode of operation the aperture force sensor device is combined with a resistive pulse sensing device and thus the combined system includes but is not limited to: a pair of electrodes, current-drive electronics, resistive-pulse sensing electronics, resistive-pulse processing electronics and the controller that typically comprise a resistive pulse sensor.

The simultaneous measurement of both aperture current and aperture force generates additional data that can provide for a more complete characterization of the analyte and the analyte-aperture interaction. It is also possible to operate the aperture force sensor sequentially, either before or after the resistive pulse measurement, especially when the operating conditions that optimize the resistive pulse measurement are different from the optimum conditions for aperture force sensing.

Where the method of the invention is used to determine the sequence of a polymer, for example to determine the sequence of a DNA molecule, the aperture may be functionalized to specifically interact with each of the bases forming the polymer chain via one or more interaction forces such that a distinct and unique membrane deflection signal is generated and can be used to identify each of the bases on the polymer chain.

Further the polymer-aperture interaction force detection may be combined with other detection methods such as resistive-pulse sensing, force spectroscopy methods, optical sensing and others to provide a robust determination of the polymer sequence.

The monomer components of the polymer molecule may also be labeled or decorated to specifically interact with an aperture such that the decorated polymer generates distinct and unique signals to enable the determination of the polymer sequence.

Objects of the invention include but are not limited to:
Providing a versatile device and method capable of sensing one or more of a wide range of interaction forces between analyte in a sample and at least one aperture in a membrane.

Detecting the presence, analyzing, monitoring and/or modifying analyte in a sample using forces generated by analyte interaction with a membrane aperture where analyte includes but is not limited to single molecules.

It is also an object of the invention to introduce the versatility of interaction force sensing to aperture-based methods and devices by providing an aperture force sensor capable of simultaneously or sequentially detecting any one, and possibly multiple, analyte-aperture interaction forces.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1A. is a schematic diagram an embodiment of the aperture force sensor of the present invention.

FIG.1B. is a schematic diagram of an embodiment of a sample cell of the aperture force sensor.

FIG.2A. is a schematic diagram an embodiment of the aperture force sensor of the present invention that also includes a resistive-pulse sensor.

FIG.2B. is a schematic diagram of an embodiment of a sample cell of the aperture force sensor that also includes the electrodes necessary for resistive-pulse sensing.

FIG.3A. shows one embodiment of the membrane assembly of the present invention including analyte flowing through an aperture.

FIG.3B. shows one embodiment of the membrane assembly of the present invention including analyte interacting with a functionalized aperture.

FIG.3C. shows one embodiment of the membrane assembly of the present invention including an analyte polymer molecule flowing through an aperture.

FIG.4. is a schematic of a multiplexed embodiment of the membrane sensor suitable for multiple analytes.

DETAILED DESCRIPTION

An aperture force sensor device according to a preferred embodiment of the present invention includes but is not limited to the following components: A membrane including at least one aperture mounted in a sample cell and sealed such that the membrane defines

the boundary between two reservoirs; and sample and analyte can translocate from the cis to the trans reservoirs only by travelling through the membrane aperture.

As shown in Figures, 1A, 1B and 3A, the membrane assembly 230 includes a membrane sensing surface 238, typically a thin film of optically reflective material that operates to reflect light to enable optical interferometric membrane deflection sensing. The membrane deflection sensor includes a light source 110, coupling optics 120, a sensor element 130 and detector 140 to convert the optical signal to an electrical signal. The sample cell 200 is supported by a precision adjustable mount to align the membrane sensing surface 238 with the membrane deflection sensor. The membrane deflection sensor output signal is directed to a signal processor 150.

The membrane assembly in Fig.3A includes but is not limited to: a support 232, a membrane 234, a membrane aperture 236, and a membrane sensing surface 238 that is capable of reflecting light. The arrow indicates the direction of flow of analyte 202 through the aperture 236.

The sample cell 200 also includes fluidic connections to direct sample and analyte to the membrane 230. As shown in Figs. 2A and 2B the sample cell 200 may also include electrodes, 270 and 280 immersed in the sample cell reservoirs and connected to a potentiostat 310 to drive and control electrokinetic flows.

As shown in Fig. 1B, the signal generator 160 may provide a driving signal to a transducer 250 located in the sample cell 200 that can apply a driving force to the membrane. Alternately the signal generator driving signal may be used to apply a driving force to sample and analyte in or near the membrane aperture. The signal generator 160 also provides an input to the signal processor 150 as a reference for the synchronous detection of amplitude and phase of membrane deflection.

As shown in Fig. 1A, the signal processor 150 accepts the membrane deflection signals as an input, accepts a reference signal from the signal generator 160 and provides an output signal to the system controller 170. The signal processor operating parameters can be optimized to aid in the identification and analysis of membrane deflection signals by use of amplification, amplitude thresholding, as well as time-domain and frequency-domain filtering and analysis.

Fluid controls 180 determine the rate of fluid flow and drive flow of sample fluid into the sample cell 200 as well as through the membrane aperture 236. Fluid controls include but are not limited to pumps to drive pressure driven flows and also potentiostats connected to electrodes immersed in the cis and trans reservoirs to enable driving electrokinetic flows.

The system controller 170 controls all subsystems via a software interface. The system controller directs fluid controls to drive flow of sample and analyte into the cis reservoir of the sample cell and also to drive translocation of sample and analyte from the cis to the trans reservoirs. The system controller 170 directs the signal generator 160 to drive membrane deflection or to drive sample and analyte motion in the modulation modes. The system controller 170 also generates control signals for the signal processor 150 and accepts the membrane deflection sensor signal as an input via the signal processor. The system controller 170 may also implement one or more feedback control loops where the membrane deflection signal is used to generate a correction signal directed to one or more transducers via the signal generator which controls membrane deflection and can maintain membrane deflection nearly constant at a predetermined value.

FIG.1A is a schematic of a preferred embodiment of the invention including a membrane deflection sensor based on optical interferometry. In this embodiment the aperture force sensor 100 includes a light source 110, coupling optics 120, sensor head 130, a sensor cell 200, a light detector 140 for detecting optical interference signals from the light waves reflected by the sensor head 130 and a membrane in the sensor cell 200. Sensor head 130

includes but is not limited to a dielectric mirror that forms an optical resonant cavity when aligned to the optically reflective membrane sensing surface 238. The optical sensor system may include a variety of components such as lenses, mirrors, beam-splitters, polarizing components, optical isolators, optical coatings, prisms, gratings, molded optical elements and associated mounting components, piezo-electric actuators. The optical components are used to direct light from the light source 110 to the sensor head 130 and to collect the reflected light from the sensor head and direct it to the light detector 140. In a preferred embodiment the light source is a stabilized Helium:Neon gas laser emitting near 633 nm. In alternate embodiments the light source 110, may be a LED, laser diode, solid state laser, gas laser, and suitable wavelengths range from the DUV to the far infrared (10 nm to 100 um). The light source 110 may emit light primarily at a single wavelength or over a range of wavelengths. The detector 140 may be a simple single-element photodetector, multi-element photodetector or a detector array, such as a charge-couple device CCD or CMOS imaging device. A single detector element can be used to monitor the signal from the sensor head 130. The optical coupling 120 assembly directs light to the sensor head 130 and also directs light back to the light detector 140. The membrane deflection is detected by optical detector 140 and delivered to a synchronous signal processor 150 which receives the signal voltage from signal generator 160 as a reference. The synchronous signal processor 150 is used to monitor both the amplitude and phase of the membrane deflection, an adjustable time constant for integration of the signal may be used to improve the signal to noise ratio. The device 100 also includes fluid controls 180 to regulate and drive sample and analyte into the sensor cell 200. A controller 170 directs all of the subsystems, including signal processor 150, signal generator 160 and fluid controls 180 as well as accepting detector signals 140 as an input.

Shown in FIGS.2A and 2B is an embodiment of the invention that combines the aperture force sensor with resistive pulse sensing to enable a more complete characterization of the aperture-analyte interaction.. In this embodiment the sample cell 200 includes electrodes 270 and 280 and the sample fluid is preferably an electrolyte such that a voltage applied to

electrodes 270 and 280 generates an ionic current flowing through the at least one aperture 236 in membrane 234. In addition the electrodes 270 and 280 are connected to a current controller 310 and pulse processor 320 to drive the ionic current and process resistive pulses due to analyte translocation through the at least one membrane aperture 236. Controller 170 directs the current controller 310 to determine the ionic current and accepts the resistive pulses from pulse processor 320 as an input.

FIG.2B shows a sensor cell assembly 200 according to one embodiment of the invention, including a membrane assembly 230; the membrane is electrically insulating and includes at least one aperture, a first compartment 210 for analyte and sample, a second compartment 220 for analyte and sample. The membrane assembly 230 also defines the boundary between the two compartments 210, 220 so that sample and analyte travels through the at least one aperture in the membrane to translocate between the two compartments. Sensor cell assembly 200 may also provide a connection 240 to a transducing element 250 to convert a signal from signal generator 160 into a driving force at the membrane. The driving force may be applied to the membrane the analyte or both. Arrows indicate the direction of flow of sample and analyte.

According to one embodiment of the invention the method is characterized by but not limited to the following steps: A membrane 230 with an aperture 236 is mounted and aligned in a sample cell 200 including cis 210 and trans 220 reservoirs and fluid controls 180 introduce sample including analyte into one of the reservoirs so that sample and analyte can interact with the aperture 236. Sample and analyte may be driven to flow through the membrane aperture using fluidic controls 180 to apply a driving force such as an applied pressure or by using a potentiostat bias voltage to generate an electric field in the case of electrokinetic flows, and also by using membrane motion, sample motion, or simply by passive diffusion.

Analyte-aperture interaction then generates an interaction force that deflects the membrane 234, 238 and deflection of the membrane is detected by a membrane deflection sensor. In a preferred embodiment, membrane deflection sensor detects membrane deflection as an optical interference signal between light from a light source 110 directed by coupling optics 120 and reflected by both the membrane sensing surface 238 and sensor element 130; detector 140 converts the optical signal to an electrical signal.

To operate in a modulation mode, controller 170 directs the Signal Generator 160 to produce a driving signal and Signal Processor 150 accepts the driving signal as an input. A driving signal from Signal Generator 160 is directed to a transducer 250 that generates a driving force at the membrane assembly 230 and may generate an oscillation of the membrane 234. Alternately a driving signal from Signal Generator 160 can generate a driving force on the sample and analyte 202 and may generate an oscillation of the sample and analyte 202 at or near an aperture 236.

Signal processor 150 and system controller 170 process, analyze, monitor and record the membrane deflection signals to characterize the analyte-aperture interaction. In one embodiment the signal processor 150 is a synchronous and phase-sensitive amplifier which receives a signal voltage from signal generator 160 as a reference. The synchronous signal processor 150 can be used to monitor both the amplitude and phase of the membrane deflection, and an adjustable time constant for integration of the signal may improve the signal to noise ratio. System Controller 170 also directs fluid controls 180 to adjust the flow of sample and analyte to optimize the detection and analysis. Fluid controls 180 may also control the concentration of analyte in the sample to optimize the detection and analysis.

Interaction forces between analyte and aperture may result from any one or more of the following phenomena, including but not limited to: mechanical and inertial forces due, for example, to particle collisions, elastic and inelastic deformation. Viscous drag forces,

hydrodynamic, frictional, lubrication, and adhesive interactions which are typically energy dissipating interactions that occur during relative motion such as the flow of sample and analyte in the aperture. Van der Waals interactions, also known as Debye induced dipole, London dispersion and Casimir forces; also ion correlation forces which are van der Waals interactions of polarizable ions. Solvation forces which may be oscillatory, alternating between attraction and repulsion. Hydrophobic forces, which are attractive hydration forces not yet completely understood. Specific binding interactions, which are the primary recognition mechanism of biological systems; specific binding of analyte to the aperture also modulates viscous drag forces by changing the effective aperture size and thus also the flow rate through the aperture. Atomic forces, also known as hard-core or steric repulsion, which are short-range forces that effectively determine molecular size and shape. Electrostatic interactions, for example in polar solvents due to surface charge or in cases where there is a charge separation mechanism. Electric forces due to charged analyte interacting with an electric field in the aperture. Magnetic forces due to magnetized analyte interacting with a magnetic field in the aperture; in this case the membrane may include a layer of magnetic material or alternately, the magnetic field may be generated by electric currents in the membrane and/or the sample. Thermal forces and stresses due to thermal effects such as thermal expansion and thermal gradients. Optical forces, for example due to radiation pressure, due to time-averaged electric field gradients in focused light beams, or indirectly due to thermal effects from optical radiation absorption. Other forces include osmotic repulsion, double-layer force, thermal fluctuation force, steric forces, undulation force and protrusion force which are associated with confinement of molecules or ions as well as capillary forces due to surface tension.

The aperture force sensor of the present invention may operate in multiple modes, including but not limited to direct modes and modulation modes.

In a direct mode, analyte translocation events are detected asynchronously by continuously monitoring the membrane deflection sensor signal as sample and analyte 202 flow through

the aperture 236. The flow of sample through an aperture 236 at a nearly constant flow rate generates viscous drag forces at the aperture 236 that result in a base-line deflection of the membrane 234, 238. The presence of analyte 202 in the aperture 236 results in an interaction force and changes membrane deflection. Typically, the presence of an analyte particle 202 in the aperture interrupts the flow of sample fluid, changing the viscous drag forces at the aperture 236. As a result, the passage of an analyte particle 202 through the aperture 236 results in an impulse that generates a membrane deflection pulse suitable for counting and sizing. In general, analyte particle translocation through the aperture generates a membrane deflection pulse with the pulse magnitude determined by the particle size relative to the aperture size, the pulse duration determined by particle translocation time which is in turn determined by particle interactions with the aperture and with the pulse frequency determined by particle concentration in the sample.

Analyte 202 interaction with the aperture 236 may also result in analyte capture or binding as shown in Fig. 3B, and this may be due to non-specific or specific binding interactions with the aperture. In one mode of operation the sample rate of flow is held constant and the analyte-aperture interaction time is measured. In another mode of operation the accumulation of bound analyte at the aperture gradually decreases the effective size of the aperture, gradually changing membrane deflection.

In a modulation mode, an external driving force is applied to the membrane, or in an alternate embodiment, a driving force is applied to the sample and/or analyte 202 to generate motion of the sample and/or analyte at or near the aperture 236.

In one embodiment, sample cell 200 shown in FIG.1B includes a transducer 250 capable of generating a driving force at the membrane. Signal Generator 160 sends a driving signal to transducer 250 via connection 240. System controller 170 directs the Signal Generator 160 to produce a driving signal that may be directed to transducer 250 and Signal Processor 150 accepts the driving signal as an input. As described below, suitable transducers may

operate based on a variety of effects including but not limited to: piezoelectric, magnetic (both permanent and electromagnets), acoustic, ultrasonic, and electrokinetic.

In a preferred embodiment a driving force can be applied to the membrane 230 when the signal generator 160 drives an electric current through a conductive membrane layer 238 that is also exposed to an external magnetic field from a nearby permanent magnet or electromagnet. In an alternate embodiment, a magnetized membrane layer 238 is exposed to an external magnetic field from a nearby permanent magnet or electromagnet. In a further embodiment a driving force can be applied to the membrane by mounting a piezo-electric element 250 near the membrane chip 230 and applying a driving voltage to the piezo-electric element. In a still further embodiment, an acoustic and/or ultrasonic transducer can be integrated into the sample cell.

The membrane driving force is under the control of signal generator 160 and the precise driving waveform amplitude and time dependence is determined by the system controller 170. The membrane driving force can vary according to a ramp, pulse, oscillation or any more general time dependence. In general the membrane driving force will result in membrane deflection that is nearly constant, slowly varying, oscillatory, pulsed or has a any suitable time dependence.

In the case of an oscillatory driving force, the resulting membrane oscillation can be used for synchronous detection to measure both amplitude and phase relative to the driving signal at a suitable frequency and enable sensitive measurement of the aperture-analyte interaction. Operating in modulation mode may also provide for more sensitive analyte detection and analysis by controlling the duration and magnitude of analyte-aperture interactions. The frequency and amplitude of the driving force can be selected to optimize the response of the sensor and may drive the membrane at one or more resonance frequencies. Measuring the phase of the membrane's periodic oscillations relative to the phase of the periodic driving signal from signal generator 160 allows for detection of

phase-shifts that typically correspond to changes in sample and analyte properties exhibiting high sensitivity to the degree of dissipation, adhesion or friction in the aperture. The accumulation of bound analyte in the aperture also changes the membrane deflection in response to a driving force. In a further embodiment it is possible to use transducer 250 to apply a DC force bias and thus control membrane tension which also determines membrane resonance frequency.

In an alternate mode of operation the driving signal from Signal Generator 160 may be used to generate a driving force on the sample and/or analyte 202 and may generate an oscillation of the sample and/or analyte 202 at or near the aperture 236. For example the electric force on charged analyte may be made to oscillate by sending an oscillating drive signal to electrodes forming a conductive layer on the membrane 238. Similarly viscous forces due to sample and analyte 202 flow through the aperture 236 may be made to oscillate by generating an oscillating flow of sample and analyte through the aperture 236. Modulated electrokinetic flows can be driven by a modulated electrokinetic voltage applied to electrodes 270, 280 immersed in sample cell reservoirs 210, 220 that control the ionic current flowing through the aperture 236.

Variable flows with increasing or decreasing flow rates and in forward or reverse directions may be used to adjust the magnitude of interaction forces. Pulsed flows may be used to apply a transient or intermittent force. Oscillating flows may operate such that the presence of analyte in or near the aperture changes the amplitude or phase of membrane oscillation. In general sample and analyte flows can have any suitable time dependence limited only by the frequency response of the membrane and the transducer generating the flow.

Force spectroscopy: In another embodiment of the method of the invention the aperture force sensor is a spectroscopy tool that measures aperture-analyte interaction over a range of driving force magnitudes. Force spectroscopy may be based on modulated membrane

driving force or may be based on modulated sample and analyte driving force. The driving force is typically varied over time, for example increasing in a linear ramp to enable characterization of the strength of the analyte-aperture interaction force.

This spectroscopy method typically produces a force vs. deflection curve, which is a plot of analyte-aperture interaction force as measured by membrane deflection as a function of the applied driving force controlled by the fluid controls 180, or by signal generator 160, or by current control 310. Membrane oscillation amplitude, or phase, or both, can also be monitored versus a ramped parameter. It is also possible to obtain a measure of the strength of analyte binding to the aperture by oscillating the membrane at a given amplitude and frequency to generate a driving force and then gradually increasing the driving force magnitude while continuously monitoring amplitude and phase of membrane deflection. By controlling the aperture-analyte interaction force over a sufficiently wide range it is even possible to modify the analyte by stretching, pulling, deforming, denaturing, separating, unraveling, unzipping, severing and cleaving (breaking bonds) the analyte.

As shown in FIG.3B, analyte-binding molecules 204 may be immobilized on membrane surface 238 and aperture 236 such that, when the membrane assembly 230 is exposed to the sample, analyte molecules 202 may specifically bind to the aperture 236 with some affinity.

FIG.3C shows an embodiment of the invention where the aperture force sensor is used to determine the sequence of a polymer, for example the sequence of a DNA molecule. In this embodiment a polymer enters an aperture 236 in membrane 234 under the influence of a driving force, for example an applied pressure, an applied electric field. As the polymer translocates through aperture 236 the elements of the polymer chain may individually interact with the aperture according to one or more of the operating methods of the invention to produce a signal that uniquely identifies the element. The polymer chain

elements may interact with the at least one aperture via one or more of the interaction forces.

The aperture may be functionalized to optimize the aperture interaction with the polymer analyte. Also, the polymer may be labeled with specifically-binding markers to optimize the aperture interaction with the polymer analyte and enable the determination of the polymer sequence.

FIG. 4 illustrates a sensor assembly 1100 in an embodiment of the invention designed for detecting multiple analytes in a sample. The sensor apparatus uses optical fibers, individual optical fibers 1110 may be mounted in a carrier, for example a multi-fiber ferrule, to provide for precise alignment of both position and angle for each fiber in the array. The array may be linear (1-dimensional) or aerial (2-dimensional). Alternately the individual fibers may be fused to form a fiber bundle. The sensor assembly 1100 is composed of the same elements described previously for the sensor head in FIGS.1 and 2, but in an array format. In one embodiment, an array of reference assemblies 1120 may be formed on the fiber end surfaces to enable membrane deflection sensing by optical interferometry. Further, an array of membrane assemblies, 230, is positioned near the reference assemblies 1120 and spaced by a gap. A spacer layer having a thickness selected for optimum sensitivity is sandwiched between the reference and membrane assemblies 1120, 230 to define a gap. Each of the membrane surfaces 234 includes at least one aperture 236 and may interact with at least one of the analytes in the sample.

Another embodiment of the invention includes membrane sensors for detecting a target substance in a reference gas or liquid, comprising a measurement membrane 234 with at least one aperture 236 functionalized with a coating that is sensitive to the target substance and a reference membrane 234 with at least one aperture 236 having a reference coating that is less sensitive to the target substance than the first coating. The measurement and reference membranes are arranged such that they can be exposed in a reference step to the

sample and in a detection step to the sample with the analyte. In this embodiment the aperture force sensor is employed for determining the difference in the deflection of the measurement membrane and the reference membrane during the reference step and the detection step.

In a further embodiment the aperture force sensor includes a microfabricated membrane array for an artificial nose setup where each membrane 234 in the array includes at least one aperture 236. Each membrane 234 is coated at or near the apertures 236 with a sensing layer that may include a polymer layer. Volatile gaseous analytes are detected by tracking the flow of analyte molecules through the apertures 236 including polymer layers. The presence of analyte in the polymer layers swells the polymer layers, changes the flow of analyte through the apertures 236 which also changes the deflection of the membranes. From the membrane deflection pattern of all membranes in the array, a characteristic 'fingerprint' of the analyte is obtained. By exposure to a sample without analyte present the membrane deflection may revert to its initial state before exposure to the analyte to enable reversible and reproducible operation of the sensor. The artificial nose setup is suitable for detection of solvents, perfume essences and beverage flavors. In a medical application, the setup detects the presence of disease biomarkers in patient breath samples.

In one application the sensor array 1100 forms a “gene chip” for detecting a plurality of different gene sequences. Each sensing element in the array has an immobilized DNA sequence designed to specifically hybridize with a complementary DNA sequence in the sample. More generally, applications of the apparatus of this invention include:

1. Screening hybridoma expression lines.
2. Characterizing antibody affinity to an antigen.
3. Characterizing and modifying binding partners, including DNA, RNA, proteins, carbohydrates, lipids, organic molecules.
4. Sequencing and modifying polymer molecules including DNA, RNA, proteins, carbohydrates, organic and inorganic molecules.

5. Characterizing and modifying the interaction of the components in a protein that participates in a multi-protein complex attached to the sensor.

6. Characterizing and modifying binding partners for a protein binding molecule attached to the sensor. Constructing a calibration curve for analyte using a set of analyte standards. Using the calibration curve to determine the analyte concentration in unknown solutions.

7. Identifying and modifying specific binding partners for single-stranded DNA or RNA attached to the sensor.

8. Single nucleotide polymorphism analysis.

9. Gas sensing, for example, an artificial "nose".

10. Measuring, monitoring, detecting and characterizing the deposition of thin films, in liquid, in air or in vacuum.

11. Measuring, monitoring, detecting, characterizing and modifying adsorption, moisture, particulate, contamination, bubble formation, surface oxidation, and corrosion in liquid, in a gaseous environment or in vacuum.

12. Detection, analysis, monitoring and modifying of disease biomarkers, virus capsids, bacteria, mammalian cells, biomembranes, biomaterials, self-assembled monolayers, molecularly imprinted polymers, langmuir-blodgett films.

Example 1: Particle counting and sizing. In one method of operation for counting and sizing analyte particles the aperture force sensor detects and measures changes in viscous drag forces produced by analyte particles suspended in a sample fluid that is translocating through an aperture.

When a dilute suspension of analyte particles flows through an aperture the passage of each individual analyte particle momentarily modulates the flow of sample fluid through the aperture. Figure 3A illustrates the translocation of analyte particles through an aperture. In this example, fluid flow through the aperture is driven by an applied voltage bias generating an electric field in the aperture that drives an ion current as well as applying a

driving force to electrically charged analyte. Alternately, fluid flow through the aperture may be driven by an applied pressure.

The steady-state flow of sample fluid through an aperture at a nearly constant flow rate generates viscous drag forces at the aperture that result in deflection of the membrane. The presence of an analyte particle in the aperture interrupts the flow of sample fluid, changing the viscous drag forces at the aperture and also changing membrane deflection. Thus the passage of an analyte particle through the aperture results in a mechanical impulse that generates a membrane deflection pulse suitable for counting and sizing. In general the number of deflection pulses indicates particle count and the amplitude of the pulse depends on the particle volume.

It is possible for more than one particle to be present within the aperture at the same time, and this is called coincidence. When this occurs only one larger pulse is generated and this may result in a lower particle count and higher particle volume measurements. However the frequency of coincidence is a statistically predictable function of analyte concentration and thus a coincidence correction may be applied to the measured data.

The membrane deflection sensor produces an electrical pulse each time a particle within the size range of the aperture passes through the aperture. The membrane deflection sensor, signal processor and system controller detect, process and record membrane deflection pulses as a known volume of sample flows through the aperture. The signal processor selects pulses, for example, to set lower and upper thresholds for pulse counting, and counts the number of pulses as a known volume of sample passes through the aperture.

A constant aperture flow rate is set by the system controller software interface and is routed to the fluid controls. Fluid controls set the flow rate of sample through the aperture by controlling an applied bias voltage or by controlling fluid pressure. Safety circuits ensure the flow-rate is set to be within the safe operating limits of the membrane.

The system controller records pulses that are present when a start/stop signal is present, and the duration of this signal is determined by the selected volume of sample passing through the aperture. The system controller directs each of the subsystems, collects the data and can then correct analyte particle count for coincidence errors. The system controller may also produce pulse height histograms to provide a statistical measure of the mean particle size and standard deviation.

Example 2: Functionalized aperture. Solid-state apertures are capable of label-free analysis of molecular binding events. It is also possible to add biochemical selectivity by anchoring molecular receptors inside the aperture. A metalized silicon nitride aperture chemically modified with nitrilotriacetic acid receptors can be used for the stochastic sensing of proteins. The reversible binding and unbinding of the proteins to the receptors can be observed in real time and single molecule binding interaction parameters can be statistically analyzed.

Nanopore fabrication: A solid state aperture can be patterned in free-standing silicon nitride (SiN) membranes supported by a silicon wafer (0.6 cm x 0.6 cm x 180 μm) using electron beam lithography and reactive ion etching. The aperture diameter may be controlled by variation of the electron beam dose. Subsequently, the SiN pores can be coated with metal by vapor deposition of 10 nm Ti and 35 nm Au.

Self-Assembled Monolayer (SAM) preparation: A mixture of two alkanethiols can be adsorbed onto a gold surface where one thiol is terminated with a nitrilotriacetic acid (NTA) group, a group that forms a tetravalent chelate with nickel Ni(II), and a second thiol that is terminated with a tri-ethylene glycol group, a group that resists protein adsorption. His-tagged proteins can bind to the SAM by interaction of the histidines with two vacant sites on nickel Ni(II) ions chelated to the surface NTA groups. The number of receptors anchored in the aperture can be adjusted by spiking the solution used for the assembly of

the alkanethiol molecular monolayer with an appropriate concentration of NTA thiols. The concentration of NTA thiols can be gradually reduced until there is a significant probability that just one receptor site is present in the aperture.

Prior to coating the apertures with SAMs, the membrane chips can be exposed to oxygen plasma for 30 seconds on both sides in a plasma cleaner in order to remove organic contaminants and to facilitate aperture wetting. The chip is mounted between two compartments of a sample cell, each filled with degassed and filtered electrolyte solution. For in situ monolayer assembly, the chip is mounted in the sample cell directly after plasma cleaning. The chambers are filled with an ethanol:water solution (1:1) containing 400 mM KCl (ethanol improves the solubility of the alkanethiols).

Two Ag/AgCl electrodes are inserted and connected to a potentiostat to set the voltage driving the flow of ions through the aperture. Membrane deflection can be monitored as the driving voltage varies to generate a deflection curve and this deflection curve can be repeatedly recorded.

For the antibody experiments, metalized and SiN apertures having diameters in the range 10 to 100 nm can be used. Mixed monolayers are prepared from 10 mol% tris-NTA solutions. After Ni-loading, the sample cell is filled with buffer and recombinant protein A is added to one compartment. After stable immobilization of the protein the sample cell is rinsed with buffer and rat IgG2a or hamster IgG is added to one compartment. The antibody – protein A interaction can then be studied under what corresponds to "mild elution conditions" in typical affinity chromatography with protein A columns.

Membrane deflection versus bias voltage measurements can be performed using a potentiostat by sweeping the applied DC bias voltage while recording the membrane deflection signal. The effective conductivity of the aperture can be obtained from a fit to the measured membrane deflection versus bias voltage curve. Comparing the measured

membrane deflection versus bias voltage curves for the same metalized aperture before and after formation of a self-assembled monolayer provides a measure of the decrease in effective pore diameter associated with the monolayer densely coating the inner aperture walls. The real-time formation of an alkanethiol monolayer inside a metalized aperture can also be monitored by the membrane deflection.

Vibrational spectroscopy: The vibrational frequency response of the membrane can be investigated using vibrational spectroscopy where the amplitude and phase of membrane oscillation is analyzed from the measured deflection response to an applied sinusoidal AC driving force. The amplitude and phase of membrane deflection can then be plotted as a function of the frequency of the applied AC voltage on a log-log scale (Bode plot) and these frequency spectra plots can be compared before and after alkanethiol monolayer assembly. Fitting the vibrational spectroscopy data to the equivalent mechanical oscillator model with dissipation enables estimation of the inertial, dissipative and restoring forces on the membrane. The decrease in aperture size upon SAM formation is expected to increase the dissipative term.

Vibrational noise analysis: Analysis of the membrane vibrational noise provides additional information about the aperture properties. Signal fluctuations can be analyzed in terms of the power spectral density (PSD) obtained by performing Fast Fourier Transformations (FFT) of membrane deflection-time traces recorded with the deflection sensor. PSDs can be compared from deflection traces of the same metalized aperture before and after modification with a SAM.

Single molecule binding events can be studied when there is a single receptor site within the pore. Protein binding kinetics can also be studied in apertures with multiple receptor sites. In this case membrane deflection is monitored as a layer of protein accumulates at multiple receptor sites lining the inside walls of the aperture. The binding of protein to receptors in a modified aperture can also be studied as the protein concentration varies.

Membrane deflection data can be analyzed to yield characteristic time constants for binding and unbinding.

Competitive binding kinetics: Imidazole has a ring structure very similar to histidine and thus competes with His-tagged proteins for binding with the Ni2+ loaded NTA receptor. Thus it is possible to study the effect of varying concentrations of imidazole on the interaction of His6-tagged protein to an alkane thiol NTA modified aperture. In the case where single molecule binding events are detected the characteristic time constants measured over a range of competitive binder concentrations can be fitted using the Hill equation to estimate a Hill coefficient (or Hill slope) that provides a measure of the cooperativity when multiple ligands bind to a receptor.

Control experiments: Binding kinetics can be compared to the case of His-tagged proteins translocating through an aperture including an alkane thiol SAM without NTA. The binding of protein without His-tag can be studied with an aperture modified to include an alkane thiol NTA self assembled monolayer. In a further control experiment protein analyte can be studied with an unmodified aperture.

Force spectroscopy: Binding kinetics can also be studied as the applied bias voltage or the applied pressure varies to control driving force on the analyte interaction with the aperture. This force spectroscopy mode of operation provides a way to study the binding kinetics.

Example 3: Single molecule detection. To study the strength of an individual neutravidin-biotin bond, biotin can be covalently linked to a single stranded DNA molecule which serves to provide the electrostatic force on the bond. The bond between neutravidin and the biotinylated ssDNA is formed in free solution well before the application of force. Individual Neutravidin-biotin-DNA complexes can be electrophoretically inserted from the cis side of a silicon nitride aperture. The size of the neutravidin protein compared to the aperture size is such that it prevents full translocation of the complex so that only the

molecule threads through the aperture. The electric field within the aperture pulls on the charged DNA, applying a force to the biotin-neutravidin bond. Under constant pulling force the receptor-ligand pair eventually dissociates and the biotinylated DNA molecule rapidly translocates to the trans side of the aperture while the uncharged neutravidin diffuses away on the cis side. The capture of individual complexes changes membrane deflection, and upon bond dissociation the membrane returns to the initial deflection state.

Nanopore fabrication: Apertures may be fabricated in silicon nitride membranes (SiNx) supported on a silicon chip frame using a focused high-energy electron beam. Silicon nitride membranes are commercially available (Ted Pella TEM windows) in a wide range of membrane sizes and silicon nitride film thickness. Typical dimensions are 50 x 50 microns square and 30 nanometer thickness. Prolonged irradiation of the SiNx membrane with an electron beam focused to a spot of a few nanometers leads to the formation of a hole via sputtering.

Nanopore cleaning: Prior to use the membrane chips are subjected to a stringent cleaning procedure to render the surface hydrophilic and facilitate wetting of the aperture.
In a typical protocol, the membrane chips are immersed in a 3:1 mixture of concentrated sulfuric acid and hydrogen peroxide (piranha solution), and kept at 95°C for 30 min. The chips are then thoroughly rinsed in filtered, degassed deionized water and immediately mounted in the sample cell containing filtered, degassed 1M KCl 10mM HEPES buffered at pH 7.0.

The membrane chip is mounted to the sample cell and sealed to electrically isolate the two fluid-filled reservoirs. Electrodes may be placed in each of the fluid-filled reservoirs to apply a bias voltage that generates an electric field at the aperture which can drive translocation of analyte molecules from the cis to trans chambers.

Sample preparation: 5′-biotinylated oligonucleotides and neutravidin, avidin or streptavidin proteins are commercially available. Biotinylated oligonucleotides may bind with neutravidin proteins in by mixing a 1:1 ratio at 10μM concentration in 1M KCl 10mM HEPES pH7.0 and allowing the components to react at room temperature.

Baseline measurements: Apertures are continuously immersed in, filtered, degassed 1M KCl 10mM HEPES pH7 and membrane deflection is monitored for an extended period of time.

Change in pore conductance due to DNA: Based on simple geometric arguments, assuming a cylindrical aperture and ignoring edge effects, the expected decrease in flow due to the presence of a DNA molecule can be estimated using the following relation:

$$\text{Delta } F / F0 \sim D_{dna}\text{^}2 / D_{aperture}\text{^}2$$

Where Delta F is the change in flow rate due to the presence of the DNA molecule in the aperture. F0 is the flow rate for the open aperture, Ddna is the effective diameter of the DNA molecule and Daperture is the effective diameter of the aperture. We expect that insertion of a DNA molecule into the aperture to reduce the aperture flow rate for any given applied bias voltage.

A change of the aperture flow rate changes the viscous drag forces at the aperture and results in a change of membrane deflection. We expect to see events with sharp transitions between the open and blocked aperture states as DNA enters the aperture and then breaking of the neutravidin-biotin bond results in rapid translocation and diffusion away from the pore of the biotinylated DNA molecule and the neutravidin protein plug. The membrane deflection signal can then be analyzed to produce deflection event histograms to characterize a population of molecules and their interaction with the aperture.

Control experiments: introducing biotinylated DNA molecules that are not coupled to neutravidin on one side of the aperture is expected to yield DNA translocation events that are much shorter than events in the force spectroscopy experiments. The detection of these DNA translocation events is limited by the frequency response of the membrane to applied forces. The membrane frequency response is typically determined by the effective membrane spring constant and the membrane dissipation due to viscous drag and other effects. Ultimately these measurements may be used to study the binding kinetics for the reaction.

What is claimed is:

1. A device for detecting, analyzing, monitoring, or modifying an analyte in a sample comprising:
    a. a support including at least one membrane;
    b. the membrane having at least one sensing surface;
    c. one or more apertures in the membrane;
    d. a sensor for sensing deflections of the at least one membrane sensing surface;

where the interaction of the analyte with one or more apertures in the membrane generates a force on the membrane that is detected by the sensor.

2. A device for detecting, monitoring, modifying or analyzing an analyte in a sample comprising:
    a. a support including at least one membrane;
    b. one or more apertures in the membrane;
    c. a sensor for sensing deflections of the membrane;
    d. a flow generator to drive translocation of sample and analyte through the aperture;

where the interaction of the analyte with one or more apertures in the membrane generates a force on the membrane that is detected by the sensor.

3. The device of Claim 2 where the flow generator utilizes pressure driven flow.

4. The device of Claim 2 where the flow generator utilizes force modulation to drive periodic translocation of sample and analyte through an aperture.

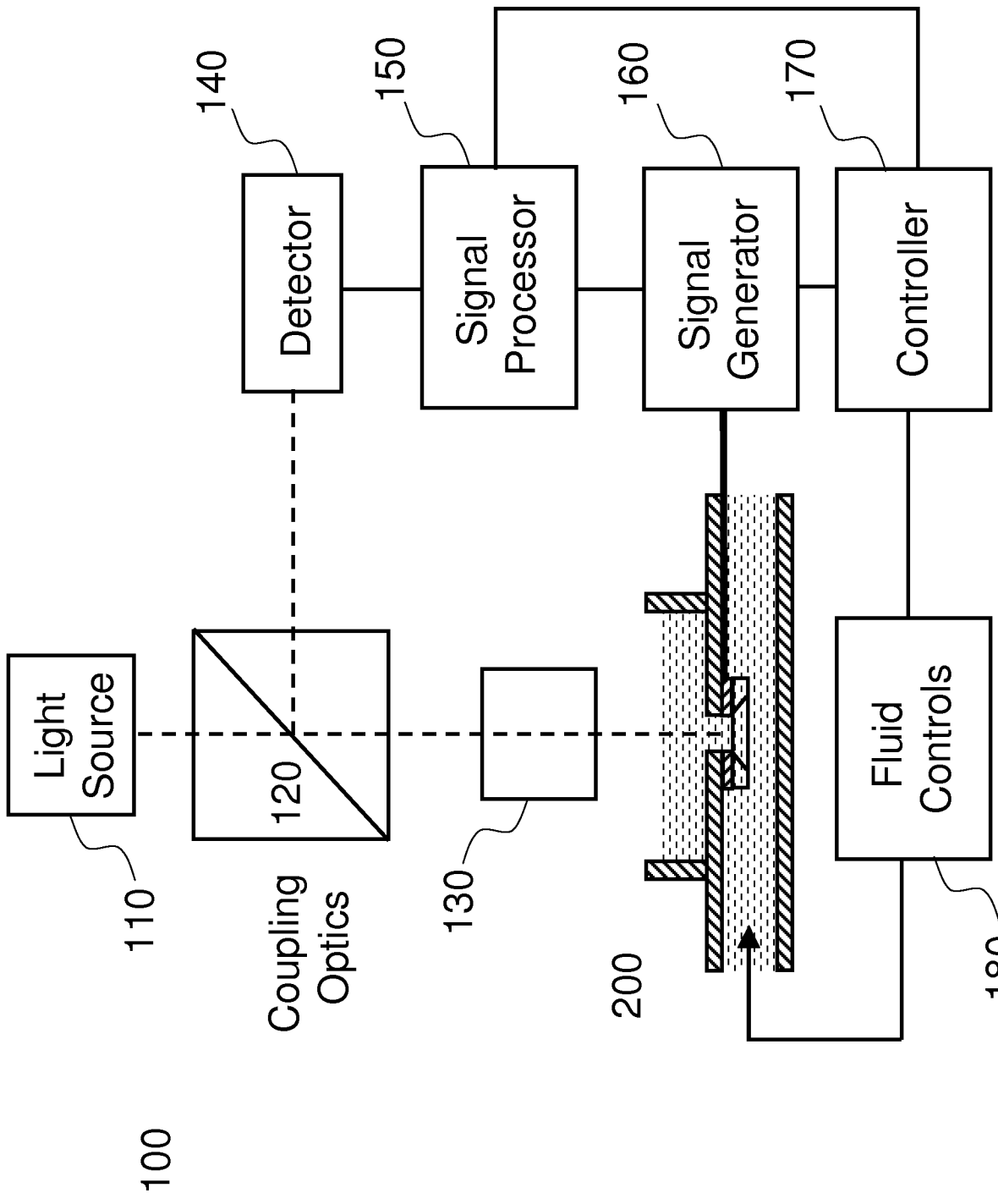
100
Light Source
110
120
Coupling Optics
130
140
Detector
150
Signal Processor
160
Signal Generator
170
Controller
200
Fluid Controls
180

Fig. 1A

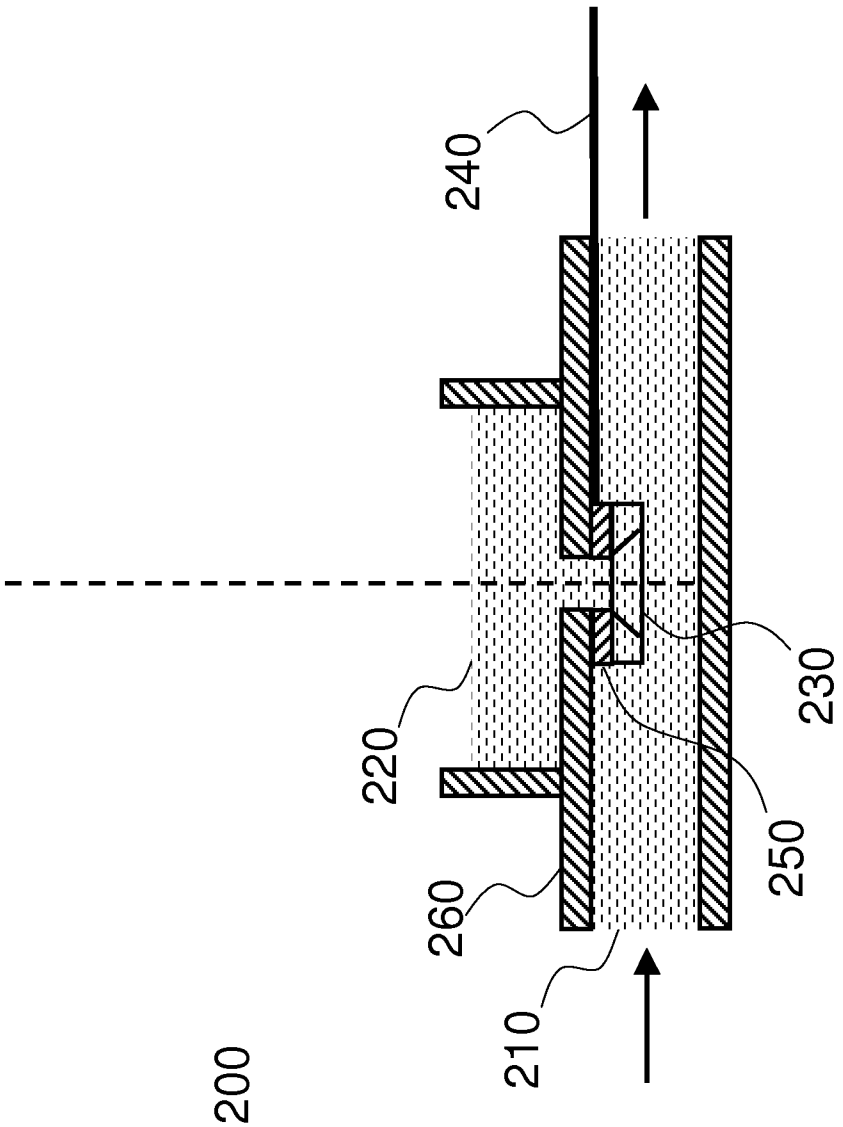

Fig. 1B

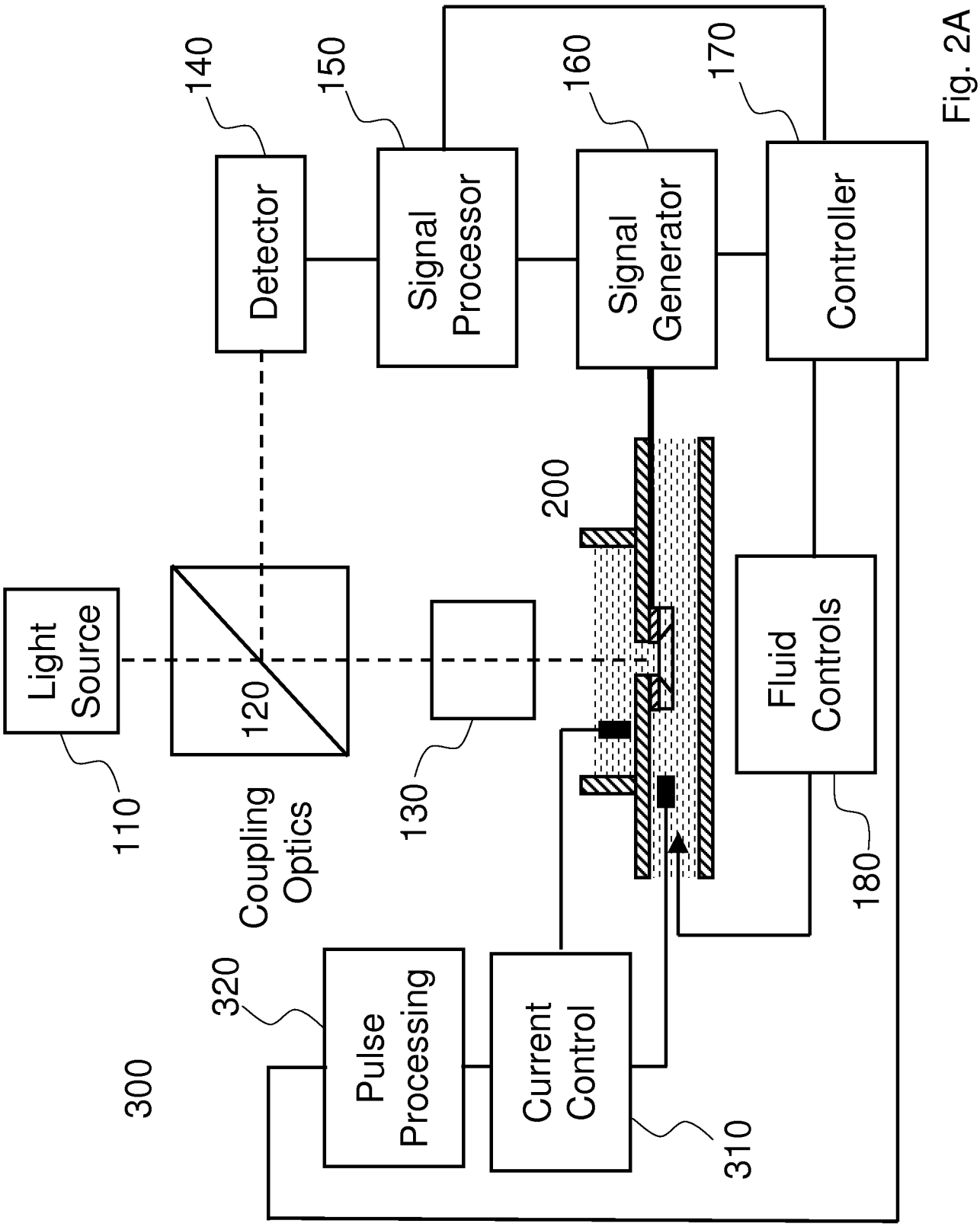
Light Source
110
120
Coupling Optics
130
140
Detector
150
Signal Processor
160
Signal Generator
170
Controller
200
Fluid Controls
180
300
320
Pulse Processing
Current Control
310

Fig. 2A

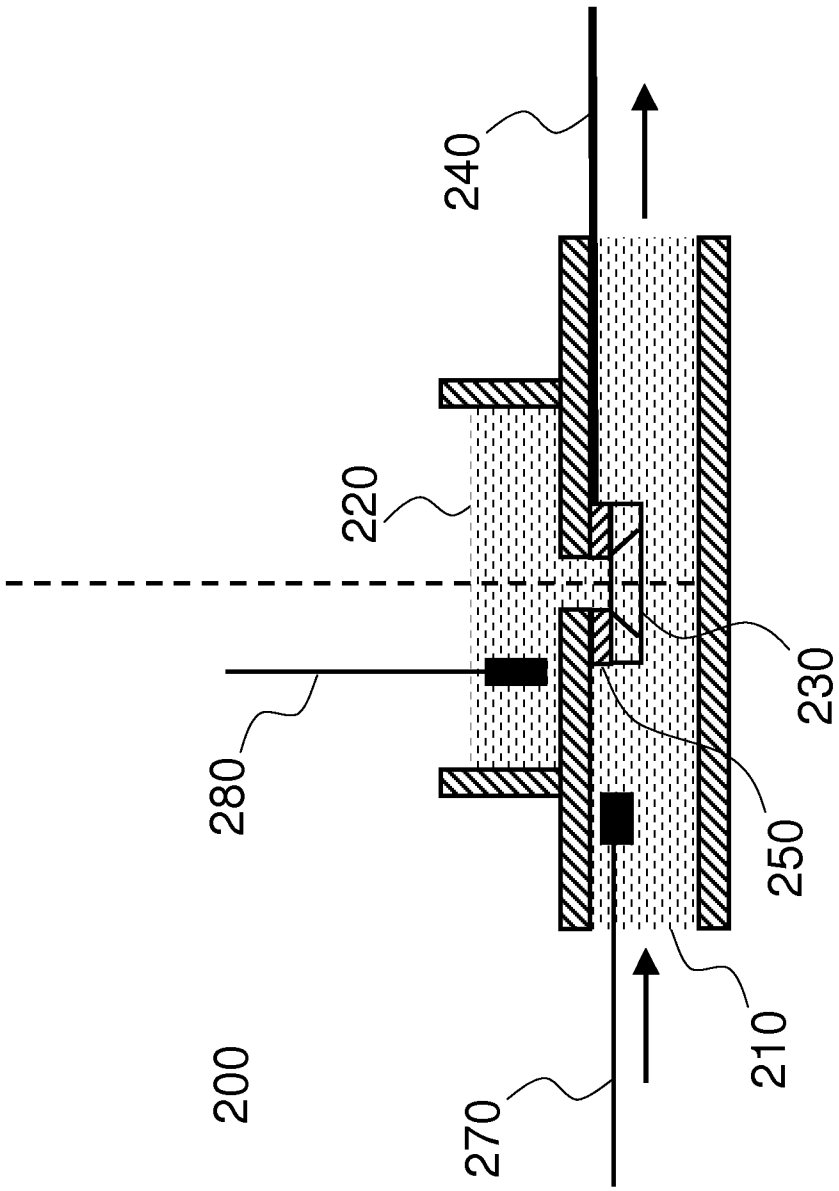

Fig. 2B

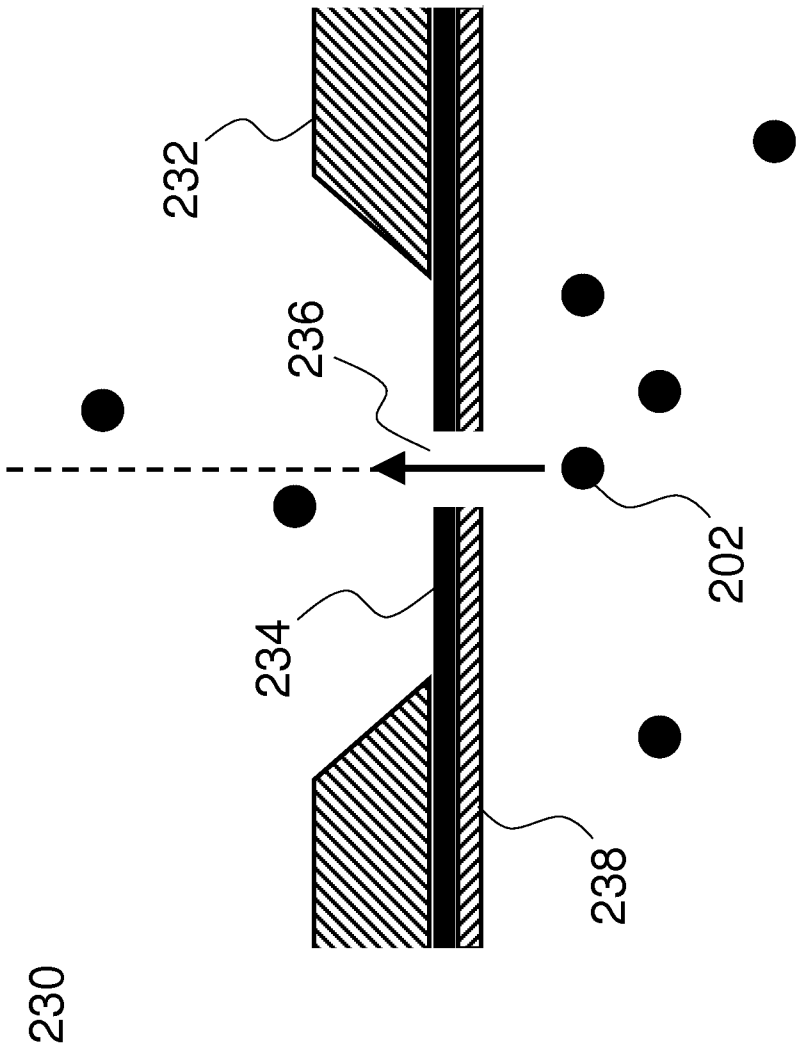

Fig. 3A

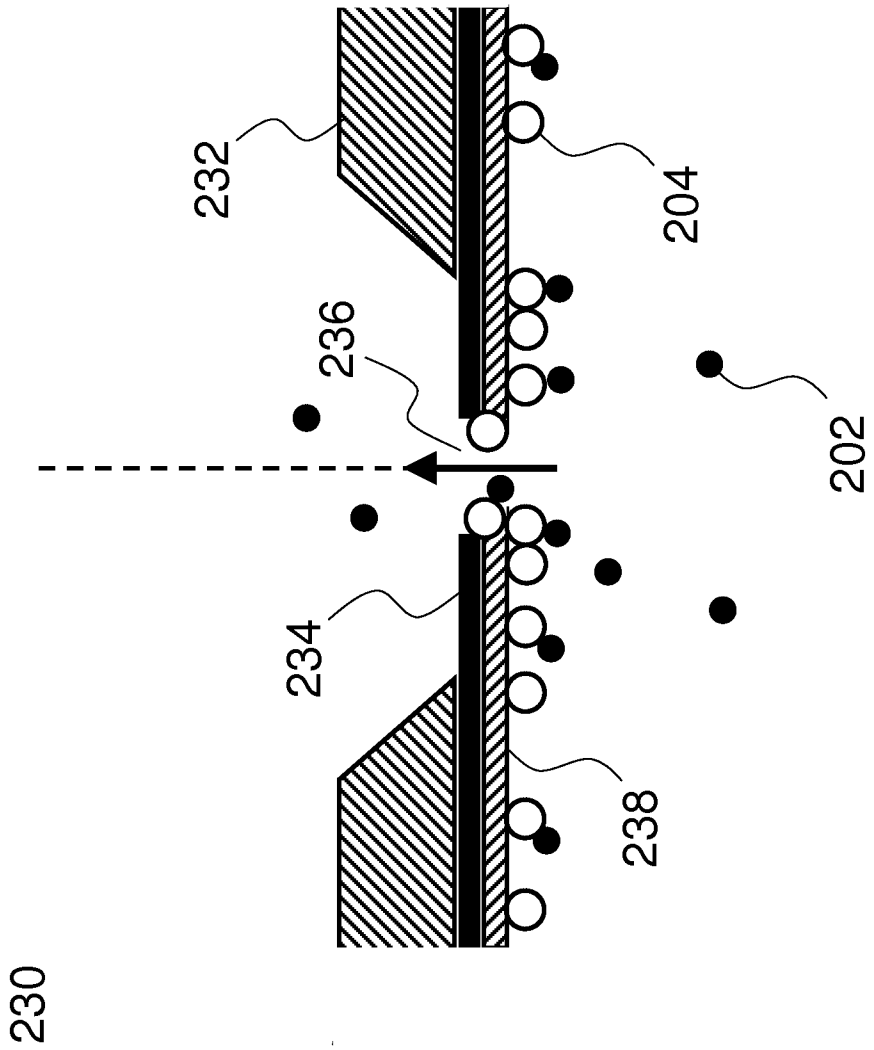

Fig. 3B

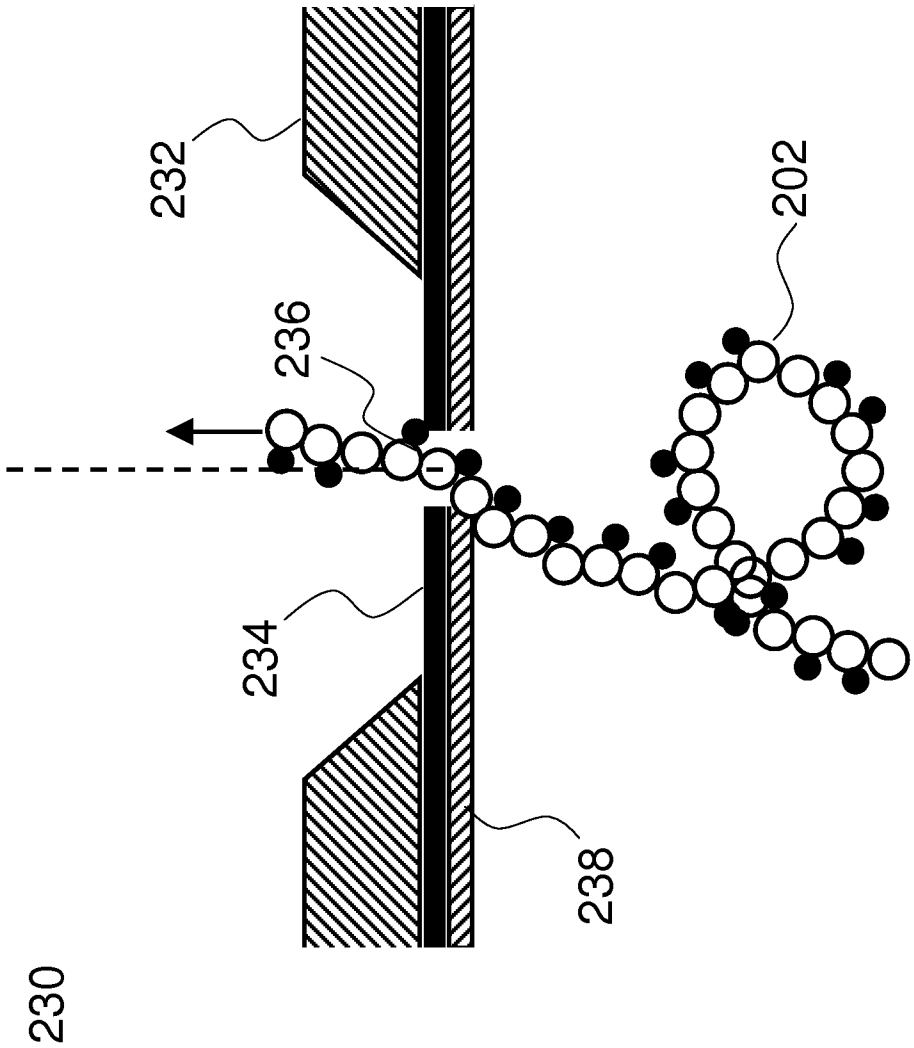

Fig. 3C

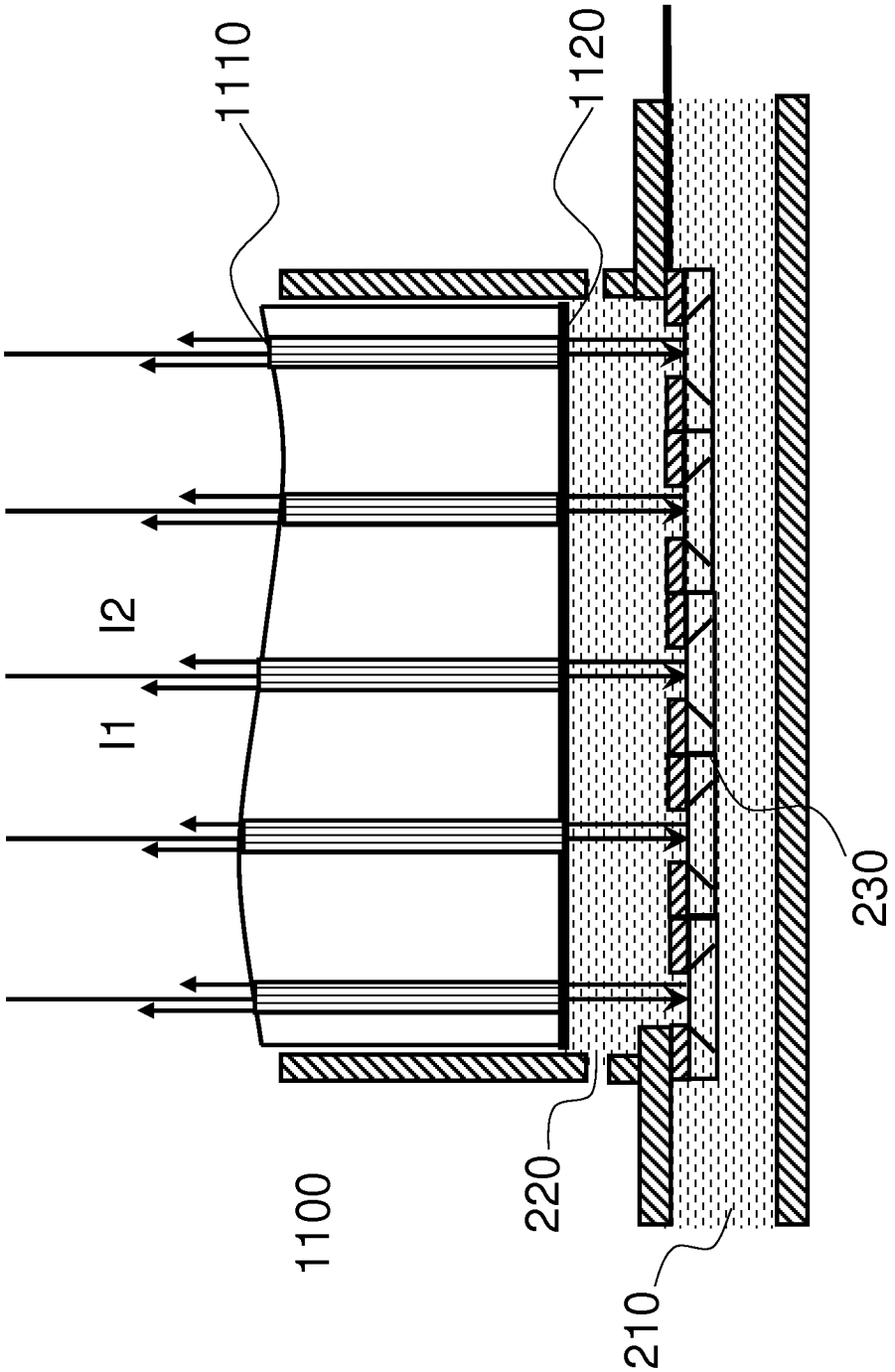

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/016992

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01B 09/02 (2014.01)
USPC - 422/81
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01B 09/00, 02, 04; G01N 21/00, 75, 29/00, 02 (2014.01)
USPC - 356/450, 480; 422/68.1, 81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - G01B 09/00, 02, 04; G01N 21/00, 75, 29/00, 02 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google, Google Scholar,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2012/0321517 A1 (GHISLAIN) 20 December 2012 (20.12.2012) entire document	1 --- 2-4
Y	US 5,167,823 A (LEIGHTON et al) 01 December 1992 (01.12.1992) entire document	2-4
A	US 2005/0254062 A1 (TAN et al) 17 November 2005 (17.11.2005) entire document	1-4
A	US 5,376,878 A (FISHER) 27 December 1994 (27.12.1994) entire document	1-4

☐ Further documents are listed in the continuation of Box C. ☐

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Date of the actual completion of the international search	Date of mailing of the international search report
17 May 2014	03 JUN 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774